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Chemical Composition and Antimicrobial Activity of Essential Oils of Lavender (*Lavandula angustifolia*) and Lavandin (*Lavandula x intermedia*) Grown in Western Romania

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Abstract

The purpose of this study was to determine the chemical composition and antimicrobial properties of essential oils (EOs) isolated from lavender (*L. angustifolia* Miller) and lavandin (*Lavandula x intermedia*) harvested in 2011 in western Romania. The essential oils, isolated by steam distillation from inflorescences arrived at full flowering stage, were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The essential oil of *L. angustifolia* Miller analyzed contained as main components caryophyllene (24.1%), beta-phellandrene (16%) and eucalyptol (15.6%), while the essential oil of *Lavandula x intermedia* contains camphor (32.7%) and eucalyptol (26.9%). The antimicrobial activity was evaluated by the Kirby-Bauer method. Antimicrobial tests showed antimicrobial activity against *Shigella flexneri*, *Staphylococcus aureus*, *E. coli* and *Salmonella typhimurium*, while *Streptococcus pyogenes* is not sensitive to the action of the two essential oils. The study revealed that essential oils isolated and analyzed from lavender (*L. angustifolia* Miller) and lavandin (*Lavandula x intermedia*) display significant bactericidal effects against microorganisms such as *Shigella flexneri*, *Staphylococcus aureus* and *E. coli* even in the absence of active principles like linalool and linalyl acetate, considered responsible for the antibacterial and antifungal properties of essential oils obtained from different species of *Lavandula*. The results suggest once again that the antimicrobial activity of EOs is a resultant of the antibacterial properties of the major and minor components in their chemical composition.

Keywords: Lavender; Lavandin; Essential oil; Steam distillation; GC-MS analysis; Antimicrobial activity

Introduction

L. angustifolia Miller or true lavender is a perennial shrub of the family *Lamiaceae* (Lis-Balchin, 2002). The main growing countries are Bulgaria and France and on smaller areas in Morocco, the former republics of Yugoslavia, Hungary, Italia, Russia, Spain, Romania, Ukraine, Turkey, and others (Zheljazkov, 2012). Their main use is the extraction of the essential oil (EO) isolated from flower heads harvested in July-August and processed fresh. The yield is between 0.6-1% (Burdock, 1998).

Lavandula × *intermedia* (lavandin) is a sterile hybrid obtained from *L. angustifolia* Miller and *L. latifolia* (L.) (spike lavender) (Raghavan, 2007). Lavandin was produced to serve as raw material to obtain EO. Its yield of EO can be five times higher than that of *L. angustifolia* (Lis-Balchin, 2002).

The EO obtained from *L. angustifolia* flowers is composed primarily of linalyl acetate, linalool, lavandulol, 1,8-cineol, lavandulyl acetate and camphor (Lis-Balchin and Hart, 1999), while the EO from *L. x intermedia* contains linalool, linalyl acetate, camphor, 1,8-cineol and borneol (Lis-Balchin, 2002).

The EO obtained from *L. angustifolia* has various medical applications due to its sedative, carminative, anti-depressive and anti-inflammatory properties (Cavanagh, 2005), while the oil isolated from *L. x intermedia* due to its high content of camphor is used mainly in the production of perfumes and soap (Lis-Balchin, 2002). In addition to these applications, both EOs are used in the food industry as natural flavorings in baked goods, alcoholic and nonalcoholic beverages, puddings etc. (Burdock, 1998).

Besides these properties numerous studies have reported that both EOs possess antimicrobial and cytotoxic

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activities against several species of bacteria (Lis-Balchin and Deans, 1997; Rota *et al*., 2004; Soković *et al.*, 2007; Hussain *et al.*, 2010; Soković *et al.*, 2010; Blazeković *et al.*, 2011; Stanojević *et al.*, 2011; Zheljazkov *et al.*, 2012). Also both EOs showed fungistatic effects against *Candida albicans, Microsporum canis, Aspergillus fumigates, Fusarium oxysporum* etc. (Blazekovic *et al.*, 2011; Stanojević *et al.*, 2011; Şerban *et al.*, 2011).

The purpose of this study is to determine the chemical composition and antimicrobial properties of EOs isolated from lavender (*L. angustifolia* Miller) and lavandin (*Lavandula* × *intermedia*) harvested in western Romania. Knowledge and understanding of the chemical composition and antibacterial properties of the EOs could contribute to accessing new natural antiseptic with applications in the food, pharmaceutical and cosmetics industries.

Materials and Methods

Raw Materials

The plant material used in the study was obtained from the experimental lots of Banat's University of Agricultural Sciences and Veterinary Medicine of Timişoara in July 2011. The lavender (*L. angustifolia* Miller) and lavandin (*Lavandula* × *intermedia*) inflorescences were harvested manually, at the maximum flowering stage, when the EO content and quality are considered the best (Guitton *et al.*, 2010; Zheljazkov *et al.*, 2012). Voucher specimens were collected from each plant that were identified and deposited in the herbarium of the Department of Agricultural Technologies, Faculty of Agronomy, Banat's University of Agricultural Sciences and Veterinary Medicine of Timişoara, Romania (Number VSNH.BUASTM-81 and VSNH.BUASTM-82).

Isolation of Essential Oils

Fresh plant material was used for the extraction of EOs. EOs was extracting by steam distillation according to the method previously described by (Craveiro *et al.*, 1976), separated by decantation then dried on anhydrous sodium sulfate and stored for the GC-MS and antimicrobial activity analyses in hermetically sealed vials at 4°C.

Gas Chromatography-Mass Spectrometry

Oil samples were analyzed by gas chromatography with a HP6890 gas chromatograph, coupled with a HP 5973 mass spectrometer. The gas chromatograph has a split-splitless injector and a capillary column Factor Four ^{TM} VF-35 ms, 35% phenylmethyl phase, 30 m × 0.25 mm, 0.25 μm film thickness. The gas chromatography conditions include a temperature range of 50 to 250°C with 4°C/min, with a solvent delay of 5 min. The temperature of the injector was maintained at 250°C. The inert gas was helium at a flow of 1.0 mL/min, and the volume of injected sample in the splitless mode was 2 μL. The MS conditions were the following: ionization energy, 70 eV; quadrupole temperature, 100°C; scanning velocity, 1.6 scan/s; weight range, 40-500 amu.

The percent composition of the essential oils was calculated. The qualitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with the mass spectrum from the spectra library NIST 98 (USA National Institute of Science and Technology software).

Determination of Antimicrobial Activity

The essential oils were tested on the following strains *Staphylococcus aureus* (ATCC 25923), *Shigella flexneri* (ATCC 12022), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Streptococcus pyogenes* (ATCC 19615).

The antibacterial activity of the essential oils was determined by using the Kirby-Bauer method (Bauer and Kirby, 1966). Briefly, the test was performed in sterile Petri dishes (100 mm diameter) containing an appropriate solid sterile media. The Gram positive and negative bacteria were cultivated on Mueller-Hinton agar (Sanimed: 20779, Romania). The surface of the plates was inoculated with 200 μL of bacterial suspension. Sterile filter paper (Whatman No. 1) discs (6 mm in diameter) containing 5, 10, 15, 20 μL of the tested essential oils were placed in the centre of the agar surface. A disc containing 10 μL of sterile broth media was used as the negative control. Two different reference antibiotics, rifampicin and tetracycline, (Oxoid, UK) amended discs, at 100 μg mL^{-1} concentrations, were used as the positive control for comparison. Each individual Petri dish was immediately covered to prevent eventual evaporation. After allowing the essential oils to diffuse across the surface for 1 h at room temperatures, the plates were sealed with sterile parafilm and incubated at 37°C for 24-48 h. The antibacterial activities of the oils and antibiotics were demonstrated by a clear zone of inhibition around the disc. The zone of inhibition was measured using electronic digital Vernier calipers. Each test was performed in triplicate on at least three separate experiments.

Statistical Analysis

Data distributions were expressed as mean values and standard deviations (SD). The Student's *t*-test was used to compare the differences between the sample mean sizes of the inhibitory zones, at the same volume, in lavender and lavandin groups. The one-way ANOVA test was used to assess mean differences between sample mean sizes of the inhibitory zones at different volumes of the same oil. All tests of significance were two-tailed. StataIC 11 statistical software (StataCorp LP, Texas, USA, version 2009) was used for data analysis. A p-value <0.05 was considered statistically significant.

Results and Discussion

The yield of EO (% v/w) was 2.75% for *Lavandula* × *intermedia* and 1.13% for *L. angustifolia* Miller; the chemical components identified are reported in Table 1.

22 components were identified in the EO obtained from *L. angustifolia* Miller, representing 99.9% of the total, the major components being caryophyllene 24.12%, beta-phellandrene 16% and eucalyptol (1,8-cineol) 15.69%. The EO of *Lavandula* × *intermedia* has as major component camphor 32.7% and eucalyptol 26.9%, 24 components being identified in this case representing 98.26% of the total.

EOs obtained from various species of *Lavandula* have

Table 1: Chemical composition of *L. angustifolia* Miller and *Lavandula* × *intermedia* oils from western Romania

No.	Compound	% of total	
		L. angustifolia Miller	*Lavandula* × *intermedia*
1.	alpha-thujene	0.4	0.38
2.	alpha-pinene	0.78	2.31
3.	camphene	1.37	1.34
4.	sabinene	0.31	0.77
5.	beta-pinene	0.94	1.84
6.	beta-myrcene	2.03	1.43
7.	carene	0.76	1.78
8.	D-limonene	2.1	3.07
9.	beta-phellandrene	16.00	3.87
10.	eucalyptol (1,8-cineole)	15.69	26.9
11.	gamma-terpinene	0.48	0.38
12.	terpineol	-	0.92
13.	terpinolene	-	1.05
14.	linalool	tr	tr
15.	terpinen-4-ol	9.57	-
16.	camphor	-	32.70
17.	borneol	5.07	7.11
18.	alpha-terpineol	6.00	1.48
19.	1,6-octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	tr	tr
20.	alpha-bergamotene	-	0.26
21.	santalene	4.5	0.94
22.	caryophyllene	24.12	4.88
23.	beta-sesquiphellandrene	0.39	-
24.	1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-	4.7	0.47
25.	bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	-	0.22
26.	naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	4.16	-
27.	naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	0.53	-
28.	alpha-bisabolol	-	4.16
Identified from total area		99.9	98.26

tr - trace (<0.05%)

Table 2: Effects of *L. angustifolia* Miller and *Lavandula* × *intermedia* oils against bacteria expressed by the mean sizes of the inhibitory zones

Test microorganism	Amount of essential oil [μL]			
	5	10	15	20
Salmonella typhimurium (ATCC 14028)	Lavandin (*Lavandula* × *intermedia*) na Lavender (*L. angustifolia* Miller) na	na na	10 ± 0.18 na	13.99 ± 0.16 na
Shigella flexneri (ATCC 12022)	Lavandin (*Lavandula* × *intermedia*) 11.41 ± 0.12 Lavender (*L. angustifolia* Miller) na	18.23 ± 0.12 na	22.05 ± 0.16 16.1 ± 0.16)	26.09 ± 0.19 20.35 ± 0.20
Staphylococcus aureus (ATCC 25923)	Lavandin (*Lavandula* × *intermedia*) 8.28 ± 0.14 Lavender (*L. angustifolia* Miller) 7.04 ± 0.14	12.12 ± 0.18 11.01 ± 0.19	16.12 ± 0.24 14.95 ± 0.2	19.92 ± 0.19 19.96 ± 0.33
Escherichia coli (ATCC 25922)	Lavandin (*Lavandula* × *intermedia*) 8.98 ± 0.19 Lavender (*L. angustifolia* Miller) 7.03 ± 0.16	10.84 ± 0.12 10.06 ± 0.16	12.74 ± 0.11 18.59 ± 0.15	21.03 ± 0.16 20.2 ± 0.23
Streptococcus pyogenes (ATCC 19615)	Lavandin (*Lavandula* × *intermedia*) na Lavender (*L. angustifolia* Miller) na	na na	na na	na na

Inhibitions are expressed in mm and include the diameter of the paper disc (6 mm). Data distributions were expressed as mean values and standard deviations (SD) (n = 9). Rifampicin and tetracycline was used as positive control; na: no activity

a very different chemical composition, the major components reported in the literature being linalool, linalyl acetate, fenchone, eucalyptol and borneol (Bouzouita *et al.*, 2005; Imeloune *et al.*, 2009; Soković *et al.*, 2010; Stanojević *et al.*, 2011; Zheljazkov *et al.*, 2012). In contrast in the composition of the EOs analyzed in this study linalool and linalyl acetate are not found or their presence is observed only in traces. Similarly the absence of these compounds has also been reported in other studies (Hui *et al.*, 2010; Abroomand Azar *et al.*, 2011).

These changes in the essential oil composition might arise from several environmental (climatic, seasonal, geographical) and genetic differences (Stanojević *et al.*, 2011).

Experimental data obtained from the evaluation of antimicrobial activity are reported in Table 2. Comparing the effects of the two EOs, for the same volumes against selected bacteria, the mean sizes of the inhibitory zones were statistically significantly higher for lavandin ($p< 0.001$, *t* test), except for *Staphylococcus aureus* (20 μL) ($p > 0.05$, *t* test) and *E. coli* (15 μL), where the results were significantly higher for lavender ($p < 0.001$, *t* test). When comparing the effects of the two EOs (at different volumes) there was a statistical significant difference for lavandin between the mean sizes of the inhibitory zones when testing against *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus* and *E. coli* ($p < 0.001$, oneway ANOVA test). No effect was observed against *Streptococcus pyogenes*. In the case of lavender statistically significant differences were recorded between the mean sizes of the inhibitory zones when testing against *Shigella flexneri*, *Staphylococcus aureus* and *E. coli* ($p < 0.001$, oneway ANOVA test). No effects were observed against *Salmonella typhimurium* and *Streptococcus pyogenes*.

The antimicrobial activity of the two EOs analyzed by us is comparable with data reported in previous studies (Hammer *et al.*, 1999; Soković *et al.*, 2007; Hanamanthagouda *et al.*, 2010; Blazekovic *et al.*, 2011; Şerban *et al.*, 2011) although they had in their composition only trace amounts (<0.05%) of linalool while linalyl acetate was not identified, these two active compounds being considered by a number of studies as possessing a strong antimicrobial effect (Dorman and Deans, 2000; Aridogan *et al.*, 2002; Soković *et al.*, 2007; De Martino *et al.*, 2009; Soković *et al.*, 2010; Blazekovic *et al.*, 2011). In the composition of the lavender EO studied we find however other components recognized for their antibacterial efficacy: caryophyllene (Oztürk *et al.*, 2009), terpinen-4-ol (Dorman and Deans, 2000; Kotan *et al.*, 2007), borneol, α-pinene, terpineol (Dorman and Deans, 2000). Camphor the major component of the Lavandin EO analyzed has antibacterial properties itself (Magiatis *et al.*, 2002; Soković *et al.*, 2007; Mahboubi and Kazempour, 2009). Moreover, it has been demonstrated that eucalyptol, one of the primary components of both EOs studied, presents antimicrobial activity against bacteria such as *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *E. coli* and *Candida albicans* (Hendry *et al.*, 2009). The same study also suggests the synergistic effect of minor components in the chemical composition of the EOs in relation to its antimicrobial activity, similar results having been reported in other studies, along with additive and antagonistic effects (Gill *et al.*, 2002; Mourey *et al.*, 2002). EOs represent complex mixtures of chemical compounds with different antimicrobial properties, and for these reasons it is very difficult to reduce their antimicrobial effect to one or several active principles (Bouzouita *et al.*, 2005).

Conclusion

The study demonstrates that lavender and lavandin EOs presents significant bactericidal effects against microorganisms such as *Shigella flexneri*, *Staphylococcus aureus* and *E. coli*, even in the absence of active principles like linalool and linalyl acetate, credited with strong antimicrobial and antifungal effects. The study suggests once again that the antimicrobial activity of the EOs is a resultant of the antibacterial properties of the major and minor components in their chemical composition. In these conditions further research is required for the elucidation of the mechanisms determining the increase of the bioactivity of Eos, but also of the synergistic relationship between their components. Explanation of these mechanisms will allow the easy accessing of new sources of natural antiseptics with applications in the food, cosmetics and pharmaceutical industries.

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